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Research Article

ANTIBACTERIAL POTENTIAL OF STEROLS OF SOME MEDICINAL PLANTS

GEETA SINGH*, PADMA KUMAR & ALKA JINDAL

Laboratory of plant tissue culture and secondary metabolites, Department of Botany, University of Rajasthan.
Email: geetsingh600@gmail.com

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ABSTRACT

Sterols extracts of *Withania somnifera, Euphorbia hirta* & *Terminalia chebula* were screened for antimicrobial activity in vitro against *Staphylococcus aureus* & *Bacillus subtilis*, *Proteus* mirabilis, *Raoultella planticola*, *Escherichia coli, Enterobacter aerogens* and *Pseudomonas* aeruginosa using 'Disc Diffusion Assay'. Minimum inhibitory concentration (MIC) of the extracts was evaluated by 'Micro Broth Dilution' method, while mimimum bactericidal/fungicidal concentration was determined by sub culturing the relevant samples. Susceptibility of the microorganisms to the extracts of these plants was compared with each other & with selected antibiotics. *B. subtilis* & *R. planticola* were the most resistant strains while the most susceptible bacterial strains were *E. coli* & *E. aerogenes*. The highest antibacterial potentials were observed for the sterols of fruits of E. hirta (IZ 21mm; AI 0.954±0.136 MIC 0.039). Total activity against *B. subtilis* & *E. aerogenes* for root sterols of *W. somnifera* was found to be same & highest (384.61ml). The present study indicates the antimicrobial potential in the tested extracts of selected plants hence may be exploited for future antimicrobial drugs.

Keywords: *W. somnifera, E. hirta, T. chebula*, Sterols, Antibacterial activity, MIC & MBC, Total activity.

INTRODUCTION

Herbal drugs have found wide spread use in many countries not only because they are easily available and are cheaper but an important reason has been the notion that they are safer than synthetic drugs. Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Many diseases have been treated with herbal medications throughout the history of mankind. The therapeutic value of medicinal plants depends upon the presence of one or more constituents possessing certain physiological and pharmacological activity. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases, simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity [1].

W. somnifera (L.) Dunal, commonly known as Ashwagandha, is an erect, grayish, undershrub (30-75 cm high) with long tuberous roots. The chemistry of *Withania* species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, and isolated [2-7]. *W. somnifera* has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, antiinflamatory agent, astringent and more recently as an antibacterial, antihyperplycemic and antitumoral, as well as to treat ulcers and senile dementia.

The plant *E. hirta* is commonly called asthma plant because of its alleged efficacy in East and West Africa in the treatment of asthma and various respiratory ailments [8-10]. The plant is used as a diuretic, febrifuge, galactogogue, purgative and vermifuge. It is reported as medication for intestinal amoebic dysentery [11, 12]. The phytochemicals in *E. hirta* include volatile oil, alkaloids, tannins, saponins and steroids [13]. The analgesic, antipyretic and anti-inflammatory properties of the plant have also been reported [14].

Terminalia chebula (Family: Combretaceae) is one of the important plants used in traditional medicine in many folkclaims and is called as "King of medicine". It is middle-sized tree, leaves are ovate, or elliptic, flowers are yellowish white, fruits are yellowish brown in colour distributed throughout in India [15]. *Terminalia chebula* contains tannin, chebulic acid, glycosides. sugar, triterpenoids, steroids and small quantity of phosphoric acid. The pharmacological activities previously reported are Antibacterial, Antifungal, Antiviral, Anticarcinogenic, Antioxidant, Adaptogenic and Antianaphylactic, Hypolipidemic, Hepatoprotective, Cardio protective, Antidiabetic Wound healing, Immunomodulatory and Chemo preventive [16].

MATERIALS & METHODS

Plant material

Different parts (fruits, leaf, stem and root) of *W. somnifera* & *E. hirta* were collected from Jaipur, (India) where as different parts (fruits, leaf, stem, stem bark) of *T. chebula* were collected from the University of Agriculture Sciences (Gandhi Krishi Vignyan Kendra, Bangalore). Voucher specimens of the plants have been deposited at the Herbarium, Department of Botany, University of Rajasthan.

Extraction of Sterols

Each of the dried materials was finely powered & defatted in a soxhlet for 24 hrs on a water bath. Each of the mixture was filtered; the residual mass was refluxed with 15% ethanolic HCL for 4 hr & the extract filtered [17]. The filtrates were then extracted separately with ethyl acetate fractions was brought to neutrality by repeated washings with distilled water & passed over sodium sulphate so as to remove traces of moisture. Neutral ethyl acetate fraction of all the samples was then dried in vacuo separately, reconstituted in chloroform, filtered, dried & weighted before subjecting it to analysis.

Selected Test Microorganisms

Pathogenic microorganisms selected for study include seven bacteria, viz., *Escherichia coli* (MTCC no. 46), *Pseudomonas aeruginosa* (MTCC 1934), *Proteus mirabilis* (MTCC 3310), *Raoultella planticola* (MTCC 2271), *Enterobacter aerogens* (MTCC 2822), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 3160). Selected microorganisms were procured from IMTECH, Chandigarh, (India). Bacterial strains were grown and maintained on "Muller-Hinton Agar Medium" (Beef extract 2.0g; Peptone 17.5g; Starch 1.5g; Agar 17.0g; in 1000ml of distilled water; Final pH 7.4±0.2) at 37±2°.

Antibacterial screening of extracts:

Disc diffusion assay (DDA) was performed for antimicrobial screening [18, 19]. MH agar (for bacteria) base plates were seeded with the standard size of bacterial, (1×10^8 CFU/ml). Sterile filter paper discs (6mm in diameter) were impregnated with 100µl of each of the extract (10mg/ml concentration) to give a final concentration of 1mg/disc, left to dry in vaccuo to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates. Each extract was tested in triplicate along with standard streptomycin (1mg/disc) for bacteria, respectively. The plates were kept at 4°C for 1h for diffusion of extract, thereafter were incubated at 37±2°C for 24 h, respectively. Zone of inhibition (IZ) or depressed growth of microorganisms was

measured and the 'Activity Index' (AI) for each extract was calculated.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC)

Minimum inhibitory concentration (MIC) was determined for plant extract showing antimicrobial activity aginst test pathogens in disc diffusion assay. Broth microdilution method was followed for determination of MIC values [20]. Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two fold serial dilution. Thereafter 100µl inoculum of standard size was added to each well. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 37±2°C for 24h for bacteria, 27±2°C for 48h for yeast and 27±2°C for 5-7 days for fungi. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extract in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms. The minimum bacterial concentration (MBC) was determined by subculturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC.

Total activity (TA)

Total activity is the volume at which test extract can be diluted without loosing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [21]. In mathematical terms it can be expressed as

RESULTS & DISCUSSION

Quantity of extracts per gram of plant material was calculated (Table 1). Root of *W. somnifera* was recorded to have maximum sterol content (15 mg/gdw) followed by leaf (13 mg/gdw).

Table 1: Sterols content of different Plants

Plant Selected	Plant part	Quantity of the extract mg/gdw
W. somnifera	Leaf	13
	Stem	5
	Root	15
	Fruits	7
E. hirta	Leaf	8
	Stem	6.5
	Root	5
	Fruits	9.5
T. chebula	Leaf	7.5
	Stem	3.5
	Stem Bark	5.5
	Fruits	5

Table 2: Antibacterial activity of sterols of selected plants by Disc Diffusion Assay

Extracts		*B. subtilis*	*R. planticola*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*E. aerogens*
W. somnifera								
Leaf	IZ	-	9.75	-	9.5	9.75	11.75	9.25
	AI	-	0.325±0.009	-	0.475±0.025	0.375±0.010	0.452±0.029	0.420±0.011
Stem	IZ	11.25	-	-	20	-	12.25	10.5
	AI	0.625±0.014	-	-	1.000±0.058	-	0.471±0.029	0.477±0.023
Root	IZ	14.25	-	10.5	11.5	-	9.75	13.25
	AI	0.792±0.042	-	0.420±0.020	0.575±0.025	-	0.375±0.029	0.602±0.012
Fruits	IZ	-	-	-	10.75	9.66	-	12.25
	AI	-	-	-	0.538±0.038	0.371±0.017	-	0.557±0.012
E. hitra								
Leaf	IZ	-	10.5	8.75	-	-	18	10.75
	AI	-	0.350±0.017	0.350±0.010	-	-	0.692±0.067	0.488±0.034
Stem	IZ	8.5	9.75	-	-	19.5	-	19
	AI	0.472±0.028	0.325±0.009	-	-	0.928±0.024	-	0.863±0.026
Root	IZ	9.25	-	-	-	9	8.83	12.5
	AI	0.514±0.014	-	-	-	0.428±0.024	0.339±0.017	0.568±0.023
Fruits	IZ	-	-	16.5	-	-	9.83	21
	AI	-	-	0.660±0.020	-	-	0.378±0.017	0.954±0.136
T. chebula								
Leaf	IZ	-	7.75	10.75	10.5	9.5	10.25	19.5
	AI	-	0.258±0.008	0.430±0.010	0.525±0.025	0.452±0.014	0.394±0.010	0.886±0.068
Stem	IZ	7.25	7.25	9.25	9.75	11.75	11.25	13.5
	AI	0.402±0.014	0.242±0.008	0.370±0.030	0.488±0.013	0.559±0.012	0.433±0.010	0.613±0.023
Stem	IZ	8.25	-	8.25	9.75	18.5	14.83	7.75
Bark	AI	0.458±0.014	-	0.330±0.010	0.488±0.038	0.880±0.041	0.570±0.017	0.352±0.012
Fruits	IZ	-	8.5	9.25	10	17	11.5	14.5
	AI	-	0.283±0.017	0.370±0.050	0.500±0.025	0.809±0.048	0.442±0.019	0.659±0.013

IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc),

AI= Activity Index (IZ developed by extract/ IZ developed by standard),

± = SEM; (-) = No activity

Extracts assayed in triplicate,

IZ of standard drug streptomycin against *B. subtilis* (18mm), *R. planticola* (30mm), *P. mirabilis* (25mm), *P. aeruginosa* (20mm), *S. aureus* (21mm), *E. coli* (26mm), *E. aerogens*(22mm).

Antimicrobial potency of sterols of selected plants were assessed by inhibition zone, activity index (Table 2), minimum inhibitory concentration & minimum bactericidal (Table 3). In the present investigation total 12 extracts were tested, and all had showen antimicrobial activity against one of the other selected pathogens. *Bacillus subtilis* & *Raoultella planticola* were found to be the most resistant microbe, against which only six extracts showed antibacterial activity. Sterols of *Euphorbia hirta* showed no activity against *Pseudomonas aeruginosa.* Most of the extracts showed bioactivity against more than two microorganisms tested. Sterols of *Terminalia chebula* exhibited good inhibitory activity against most of the pathogens. Among all the bacteria tested, *Enterobacter aerogens* was found to be most sensitive, and inhibition zone produced by extract (sterols of fruits of *E. hirta*) against this pathogen was maximum (IZ 21mm; AI 0.954±0.136) with low MIC values (0.039mg/ml). Sterols of fruits of *E. hirta* also exhibited high antibacterial activity against *B. subtilis* (IZ 16.5mm; AI 0.660±0,020), with modest activity against *E. coli* (IZ 9.833; AI 0.378±0.017). However *E. hirta* stem sterols showed maximum activity against *S. aureus* (IZ 19.5mm; AI 0.928±0.024) followed by stem sterols of *T. chebula* (IZ 18.5mm; AI 0.880±0.041). In *Withania somnifera* maximum activity was showen by sterols of stem against *P. aeruginosa* (IZ 20mm; AI 1.000±0.058).

The range of MIC & MBC of extracts recorded was 0.039-0.625mg/ml & 0.039-1.25mg/ml, respectively. Lowest MIC value (0.039mg/ml) was recorded against *P. mirabilis, P. aeruginosa, S. aureus, E. coli, E. aerogens* where as against *B. subtilis* & *R. planticola*, lowest MIC observed was (0.078mg/ml) indicating significant antimicrobial potential of extracts. Bactericidal effect of sterols was recorded against *B. subtilis, P. mirabilis, P. aeruginosa, S. aureus, E. coli* and *E. aerogens*. Sterols of selected plants were recorded to be bacteriostatic against *R. planticola*. TA (Total activity) as a measure of potency was also determined (Table 4). Maximum TA value calculated was 384.61 against *B. subtilis* & *E. aerogens.*

Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world [22]. Even though pharmaceutical companies produce number of new antibacterial drugs, but gradual resistance to these drugs has increased which is matter of global concern besides synthetic drugs are normally associated with side effects (hypersensitive, immune suppression etc). Use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. Present study is an effort towards this direction. Results reveal that all the tested extracts of selected plants exhibited growth inhibitory activity against one of the other bacterial strains selected.

Hence sterols of selected three plants (*T. chebula, W. somnifera* and *E. hirta*) can be exploited for future antimicrobial formulations.

Table 3: MIC and MBC values of selected plants against bacterial pathogens

Extracts		*B. subtilis*	*R. planticola*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*E. aerogens*
W. somnifera								
Leaf	MIC	-	0.156	-	0.156	0.312	0.078	0.312
	MBC	-	0.312	-	0.312	0.625	0.156	0.625
Stem	MIC	0.078	-	-	0.039	-	0.078	0.156
	MBC	0.156	-	-	0.039	-	0.312	0.312
Root	MIC	0.039	-	0.078	0.078	-	0.156	0.039
	MBC	0.078	-	0.156	0.156	-	0.312	0.156
Fruits	MIC	-	-	-	0.078	0.156	-	0.078
	MBC	-	-	-	0.312	0.312	-	0.156
E. hitra								
Leaf	MIC	-	0.156	0.625	-	-	0.078	0.156
	MBC	-	0.078	1.25	-	-	0.078	0.312
Stem	MIC	0.625	0.312	-	-	0.078	-	0.039
	MBC	1.25	0.625	-	-	0.078	-	0.078
Root	MIC	0.312	-	-	-	0.312	0.312	0.078
	MBC	0.312	-	-	-	0.625	0.625	0.156
Fruits	MIC	-	-	0.078	-	-	0.156	0.039
	MBC	-	-	0.078	-	-	0.312	0.039
T. chebula								
Leaf	MIC	-	0.625	0.039	0.078	0.156	0.156	0.039
	MBC	-	1.25	0.078	0.156	0.625	0.312	0.078
Stem	MIC	0.625	0.312	0.078	0.156	0.078	0.078	0.156
	MBC	1.25	1.25	0.156	0.156	0.156	0.156	0.312
Stem	MIC	0.312	-	0.625	0.312	0.039	0.039	0.625
Bark	MBC	1.25	-	1.25	0.625	0.039	0.078	1.25
Fruits	MIC	-	0.078	0.156	0.156	0.039	0.039	0.156
	MBC	-	0.312	0.625	0.625	0.078	0.156	0.156

MIC = Minimum Inhibitory Concentration (mg/ml); MBC = Minimum Bactericidal (mg/ml)

Table 4: Total activity of Sterols of selected plants against bacterial pathogens

Extracts	*B. subtilis*	*R. planticola*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*E. aerogens*
W. somnifera							
Leaf	-	83.33	-	83.33	41.66	166.66	41.66
Stem	64.10	-	-	128.20	-	64.10	32.05
Root	384.61	-	192.30	192.30	-	96.15	384.61
Fruits	-	-	-	89.74	44.87	-	89.74
E. hitra							
Leaf	-	51.28	12.8	-	-	102.56	51.28
Stem	10.4	20.83	-	-	83.33	-	166.66
Root	16.02	-	-	-	16.02	16.02	64.10
Fruits	-	-	121.79	-	-	60.89	243.58
T. chebula							
Leaf	-	12	192.30	96.15	48.07	48.07	192.30
Stem	5.6	11.21	44.87	22.43	44.87	44.87	22.43
Stem Bark	17.62	-	8.8	17.62	141.02	141.02	8.8
Fruits	-	64.10	32.05	32.05	128.20	128.20	32.05

Total activity= Extract per gram dried plant part MIC

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